related increase across groups. The authors concluded that oral exposure to trichloroethylene at doses below those that cause maternal toxicity did not affect fertility or pregnancy outcome and that the accumulation of trichloroethylene and trichloroacetic acid in ovaries, adrenal glands, and uteri had no impact on mating success. No information on any structural anomalies was provided. The lack of any effects on offspring survival argues against any functional consequences, except for the preferential loss of offspring females at birth, which remains unexplained.

Berger and Horner (2003) exposed female rats to a number of male reproductive toxicants, including trichloroethylene and tetrachloroethylene, and assessed the fertilization of their oocytes by sperm from unexposed males in vitro. Female Simonson albino rats were administered drinking water containing trichloroethylene at 0.45% for 2 weeks. They were induced to ovulate, and their oocytes were incubated with semen from unexposed male rats from the same colony. Trichloroethylene significantly reduced fertilizability of the oocytes (46% versus 57% in the vehicle control females [$P < 0.005$]). Trichloroethylene also significantly reduced the number of penetrated sperm per oocyte (0.70 per oocyte versus 0.81 per oocyte in the vehicle control [$P < 0.05$]). Oocytes from trichloroethylene-exposed females also had reduced ability to bind sperm plasma membrane proteins compared with oocytes from the vehicle controls ($P < 0.05$).

Tetrachloroethylene, administered in drinking water at 0.9%, reduced the percentage of females ovulating compared with the vehicle values (53% versus 78%, $P < 0.05$). There were no effects on oocyte fertilizability or on the number of penetrated sperm per oocyte. Nose-only inhalation exposure of females to tetrachloroethylene at 1,700 ppm for two 1-hour periods per day for 2 weeks slightly reduced the fertilizability of oocytes (from 85% to 80%, $P < 0.05$). The number of penetrated sperm per oocyte was more obviously reduced (1.6 for exposed females versus 2.5 for unexposed females). There were no clinical signs of toxicity. Berger and Horner (2003) view their work as the first documented in vivo effect on oocyte fertilizability by a reproductive toxicant for a female mammal (for trichloroethylene and tetrachloroethylene). Because there is evidence that both compounds are male reproductive toxicants via a mechanism that does not involve the endocrine system, these results in females support their hypothesis.

ANIMAL STUDIES OF DEVELOPMENTAL TOXICITY

Avian and Mammalian Species

The avian explant model is used for descriptions and mechanistic studies of heart development and teratogenesis (as well as for other organ system development) because of the conservation of developmental stages and perturbations across vertebrates, especially birds and mammals, and because of the access to, and visibility of, developing avian structures in ovo and in vitro. Studies of trichloroethylene in the in ovo development of chicks have reported increased mortality and developmental defects, including lighter pigmentation, edema, evisceration (failure of abdominal wall closure, gastroschisis), decreased growth, beak malformations, club foot, and patchy feathering (Bross et al. 1983). Loeber et al. (1988) reported cardiac defects that involved inflow and outflow abnormalities, including septal defects, conotruncal abnormalities, atrioventricular canal defects, hypoplastic ventricle, and abnormalities in cardiac muscle.

Dorfmueller et al. (1979) compared the effects of timing of exposure to trichloroethylene on reproductive outcomes of female Long-Evans hooded rats exposed to trichloroethylene by inhalation at concentrations of 1,800 ± 200 ppm. Groups of rats were exposed before mating only, during pregnancy only, and throughout pre-mating, mating, and pregnancy. There were no effects of any exposure paradigm on maternal body or liver weights or on pre- or post-implantation loss, litter size (live, dead, resorbed, total), resorption rate, fetal body weight, or sex ratio. Fetal skeletal anomalies (predominantly incomplete ossification of sternum) and soft tissue anomalies (displaced right ovary) were significantly increased only in the group exposed during gestation. The investigators considered these effects to be evidence of developmental delay in maturation rather than teratogenesis. Variable effects were observed in the mixed function oxidase enzyme assay which did not correlate with treatment or pregnancy state. However, when the two groups with and the two groups without gestational exposure were compared, a significant increase in ethoxycoumarin dealkylase was associated with gestational exposure. Behavioral evaluation of the pups indicated no effect of treatment in general motor activity in any groups at any age. A reduction in postnatal body weights was observed in the offspring of mothers with pregestational exposure. The authors concluded that No results indicative of treatment-related maternal toxicity, embryotoxicity, serum teratogenicity, or significant behavioral deficits were observed in any of the treatments groups (Dorfmueller et al. 1979, p. 153).

Schwetz et al. (1975) exposed timed-pregnant Sprague-Dawley rats and Swiss Webster mice to trichloroethylene by inhalation at a concentration of 300 ppm (twice the maximum allowable excursion limit for human industrial exposure defined by the American Conference of Governmental Industrial Hygienists) for 7 hours/day on gestational days 6-15. No effects from trichloroethylene were found in rat or mouse dams (except for a statistically significant 4-5% reduction in maternal body weights in rats) or conceptuses using standard Segment II developmental toxicity assessments, including pre- and post-implantation loss, litter size, fetal body weight, crown-rump length, and external, visceral, skeletal, and total malformations and variations.

Smith et al. (1989, 1992) studied the trichloroethylene metabolites trichloroacetic acid and dichloroacetic acid in pregnant Long-Evans rats and found that both metabolites reduced body weight and growth and produced cardiac defects. The most common findings after treatment with trichloroacetic acid were levocardia (at 330 mg/kg/day and greater) and interventricular septal defect (800 mg/kg/day and greater). With dichloroacetic acid, resorptions significantly increased at 900 mg/kg/day; the most common cardiac malformations were a defect between the ascending aorta and right ventricle (at 140 mg/kg/day and greater), levocardia (at 900 mg/kg/day and greater), and intraventricular septal defect (at 1,400 mg/kg/day and greater). Thus, trichloroacetic acid appears to be more potent than dichloroacetic acid in causing cardiac teratogenicity, although both compounds exhibited dose-response relationships. The authors did not find a no-observed-adverse-effect level for trichloroacetic acid, but they concluded that the no-observed-adverse-effect level for the developmental toxicity of dichloroacetic acid in rats was 14 mg/kg/day (Smith et al. 1992).

A follow-up series of four studies on dichloroacetic acid were performed to determine the most sensitive period of development and to further characterize the heart defects (Epstein et al. 1992). The heart defects found were predominantly high interventricular septal defects and, less commonly, interventricular septal defects. The authors suggested that high interventricular septal defects are a specific type of defect produced by a failure of proliferating interventricular

septal tissue to fuse with the right tubercle of the atrioventricular cushion tissue. In the proposed model, of the three foramina (primum, secundum, and tertium) initially present, a single interventricular foramen is eventually obliterated. They also proposed that dichloroacetic acid interferes with closure of the interventricular foramen tertium, allowing the aorta to retain its embryonic connection to the right ventricle. In these studies, disruption of these septation processes did not affect the aortic connection with the left ventricle. The authors rightly questioned why dichloroacetic acid has a selective effect on fetal cardiogenesis. They speculated that perhaps the dichloroacetic acid target is a unique cell type at a unique time the biochemical differentiation of cardiocytes (Epstein et al. 1992).

Dawson et al. (1990) reported that continuous delivery of trichloroethylene into the gravid uteri of Sprague-Dawley rats resulted in increased incidence of fetal heart malformations (on a fetal basis that is, number of fetuses with heart defect[s]/number of fetuses examined). The incidence was 9% with trichloroethylene at 15 ppm and 14% with trichloroethylene at 1,500 ppm, compared with a 3% incidence in the control group (an approximately 36% increased incidence at a 100-fold increase in exposure).

In another study with a more conventional experimental design, Dawson et al. (1993) exposed Sprague-Dawley rats to trichloroethylene (1.5 or 1,100 ppm) or dichloroethylene (0.15 or 110 ppm) in drinking water before pregnancy, during pregnancy, and both before and during pregnancy. They found no differences among groups in the percentage of live births, uterine implants, or resorptions. There were also no differences among groups in congenital abnormalities other than cardiac defects. However, it is unclear how completely teratogenesis was evaluated. Of the 238 fetuses in the control group, 3% had cardiac defects (2.5% of the more than 600 fetuses in control groups in this and previous studies exhibited cardiac defects). In this study, the high concentrations of trichloroethylene and dichloroethylene were 733% higher than the low concentrations, but the increased incidence of cardiac malformations was only 13% with trichloroethylene and 12% with dichloroethylene. The dose-response curve was extraordinarily flat.

To evaluate the proximate teratogen(s) responsible for the fetal cardiac malformations associated with trichloroethylene and dichloroethylene, Johnson et al. (1998a,b) tested several metabolites of the two compounds in Sprague-Dawley rats. They found an increased incidence of cardiac malformations with trichloroacetic acid at a concentration of 2,730 ppm (10.53% versus 2.15% in the cumulative control group; $P = 0.0001$ for fetuses and $P = 0.0004$ for affected litters). The cardiac malformations included atrial septal defect, perimembranous ventricular septal defect, pulmonary artery hypoplasia, aortic hypoplasia, mitral valve defect, muscular ventricular septal defect, and pulmonary valve defect. Increased cardiac defects were not found with the other metabolites tested (monochloroacetic acid, trichloroethanol, trichloroacetaldehyde, dichloroacetaldehyde, carboxymethylcysteine, and dichlorovinyl cysteine). The metabolite dichloroacetic acid was not evaluated in this study. The investigators asserted that the low number of cardiac defects found in the metabolite groups (other than trichloroacetic acid) does not preclude teratogenicity, because the study might not have had enough statistical power to detect an effect. They also asserted that the study does not prove that trichloroacetic acid is a human cardiac teratogen. Limitations associated with the study include discrepancies in the number of affected hearts and fetuses reported in the paper and failure to disclose that the control group was not concurrent.

Johnson et al. (2003) sought to identify a threshold dose of trichloroethylene in rats. They reclassified the data reported by Dawson et al. (1993) and assessed them with information

on two lower test concentrations (0.0025 and 0.25 ppm). The authors concluded that their analysis identified a threshold level of less than [0.25 ppm trichloroethylene] above which rats exposed to increasing levels of [trichloroethylene] during pregnancy have increasing incidences of cardiac malformations in their fetuses.

Fisher et al. (2001) also evaluated trichloroethylene (500 mg/kg/day) and the metabolites trichloroacetic acid (300 mg/kg/day) and dichloroacetic acid (300 mg/kg/day) for teratogenicity. The two metabolites produced significantly reduced fetal body weights on both a per fetus and a litter basis. They found no statistically significant increases in the incidence of fetal heart malformations by litter or fetus for trichloroethylene or the two metabolites. The incidences were 4.5%, 3.3%, and 4.7% for trichloroethylene, trichloroacetic acid, and dichloroacetic acid, respectively. Interestingly, the rate of cardiac malformations observed in the treatment groups, although not different from the concurrent controls, was similar to those reported in the treatment groups by Johnson et al. (1998a,b) and Dawson et al. (1993). Of note, the frequency of the abnormalities in the soybean oil control group were higher in this study (6.5%) than in the control groups (individually and grouped) of Johnson et al. (1998a,b, 2003) and Dawson et al. (1993). Such a difference would decrease the power to detect a difference.

Collier et al. (2003) studied the effects of trichloroethylene, dichloroethylene, and trichloroacetic acid on gene expression in rats during cardiac development. They found up-regulated transcripts including genes associated with stress response (*Hsp70*) and homeostasis (several ribosomal proteins). Down-regulated transcripts included extracellular matrix compounds (GPI-p137 and vimentin) and Ca^{2+} responsive proteins (Serca-2 Ca^{2+}-ATPase and β-catenin). Down-regulated sequences appear to be associated with cellular housekeeping, cell adhesion, and developmental processes. Two possible markers for fetal trichloroethylene exposure were Serca-2 Ca^{2+}-ATPase and GPI-p137.

Collier et al. (2003) considered that cardiac insufficiency is a plausible explanation for the reduced incidence of reported malformations from in utero exposure to trichloroethylene. They argued that a lack of exposure studies in rats and mice terminated before embryonic day 18 (mice) or day 21 (rats) would exclude findings of gross cardiac defects inconsistent with life that could be identified only early in gestation (these conceptuses would die before term). The authors therefore associated the limited reports of cardiac defects associated with [trichloroethylene] exposure with timing of the analysis, not an absence of cardiac-related effects from exposure (Collier et al. 2003, p. 495). However, the timing of necropsy and fetal heart examinations in rodent models has been the same for researchers reporting rodent fetal heart malformations and researchers not reporting those effects. Consistent with the changes in gene regulation that Collier et al. observed, trichloroethylene and trichloroacetic acid, but not trichloroethanol or chloral, inhibited in vitro gap-junction-mediated intercellular communication, an important part of cellular adhesion and cardiac development (Klaunig et al. 1989).

Coberly et al. (1992) used the mouse embryo chimera assay to evaluate the effects of trichloroethylene on preimplantation embryos. Superovulated female CD-1 (Swiss) mice were treated with trichloroethylene intraperitoneally (0, 0.01, 0.02, or 10 μg/kg) or by gavage (0, 0.1, and 1.0 μg/kg; 0, 48.3, and 483 mg/kg) when the embryos were traversing the pronuclei stages of development. Embryos were flushed from excised oviducts and scored for numbers, embryonic stages, and viability for each female. The stages included degenerate and 1-cell embryos, and 2- and 4-cell embryos. All 4-cell embryos from females within a dose group were pooled, and the chimeras constructed from them. No treatment-related effects were seen on the total number of

embryos recovered from the oviducts of trichloroethylene-treated females, and no significant cell proliferation decreases were observed for any of the experimental chimeric embryos.

Other Species

Relevant toxicity studies have been performed in animal models other than rodent and avian species, including daphnids and amphibians. Niederlehner et al. (1998) evaluated the reproductive response of the daphnid *Ceriodaphnia dubia* to industrial chemicals alone and as mixtures of trichloroethylene, benzene, toluene, ethylbenzene, *m*-xylene, and tetrachloroethylene. The reproductive median inhibition concentration was 82 μM for trichloroethylene and 4 μM for tetrachloroethylene. Mixtures of trichloroethylene, benzene, and toluene had effects at concentrations below their individual lowest-observed-effect levels. In addition, observed responses to mixtures differed significantly from that predicted from a concentration-addition model, with the predicted relationship overestimating mixture toxicity (Niederlehner et al. 1998).

The Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) has been used to study the developmental toxicity of trichloroethylene. Trichloroethylene has tested positive in this assay (Fort et al. 1991, 1993). The trichloroethylene metabolites dichloroacetic acid, trichloroacetic acid, trichloroethanol, and oxalic acid have also tested positive in the FETAX assay, but each was significantly less toxic than trichloroethylene. It was suggested that trichloroethylene oxide, a highly embryotoxic epoxide intermediate formed from mixed-function oxidation-mediated metabolism, might play a significant role in the developmental toxicity of trichloroethylene (Fort et al. 1993).

Embryonic larvae of four North American amphibian species wood frogs (*Rana sylvatica*), green frogs (*Rana clamitans*), American toads (*Bufo americanus*), and spotted salamanders (*Ambystoma maculatron*) were exposed to tetrachloroethylene and its metabolites, trichloroethylene and *cis*- and *trans*-dichloroethylene. Tetrachloroethylene and trichloroethylene were teratogenic to amphibian embryos, with median effective concentrations (EC_{50}s) (malformations) of 12 mg/L for tetrachloroethylene in wood frogs and 40 mg/L for trichloroethylene in green frogs; these concentrations did not affect embryonic survival. American toads were less sensitive, with no EC_{50} for developmental abnormalities attained at the highest test concentrations (tetrachloroethylene at 45 mg/L and trichloroethylene at 85 mg/L) (McDaniel et al. 2004).

In Vitro Studies

Saillenfait et al. (1995) used rat whole embryo cultures to retain embryonic structural integrity and to preclude the presence of maternal absorption, distribution, metabolism, and excretion. They exposed explanted Sprague-Dawley embryos (gestational day 10) to trichloroethylene, tetrachloroethylene (with or without microsomes), or one of four chlorinated compounds (trichloroacetic acid, dichloroacetic acid, chloral hydrate, and trichloroacetyl chloride). They found concentration-dependent decreases in growth and differentiation indices and increases in the incidence of morphologically abnormal embryos with all the test chemicals. Trichloroethylene and tetrachloroethylene produced qualitatively similar patterns of

abnormalities, whereas their metabolites produced distinguishable dysmorphic profiles. The presence of hepatic microsomal fractions in the culture medium enhanced embryotoxic effects. Embryo lethality was defined as loss of heartbeat; the percentage of explants with a heartbeat ranged from 36% with trichloroethylene and 43% with tetrachloroethylene to 86% and 100%, respectively, in the presence of the microsomal biotransformation system. Heart defects were not mentioned. All treatments at higher doses caused a treatment-related reduction in the first branchial arch, and an abnormal brain was the most prominent effect noted. Incomplete closure of the neural tube was also noted. Chloral hydrate caused pericardial dilation (at 2 mM, with 100% embryo lethality at 2.5 mM). With respect to embryo lethality, the order of potency for metabolites was chloral, trichloroacetyl chloride, dichloroacetic acid. The dose-response curve for embryo lethality was steep. Trichloroethylene at 15 mM caused malformations but no embryo deaths, but 30 mM was 90% embryo lethal. Tetrachloroethylene caused 10% embryo lethality at 7.5 mM and 83.5% embryo lethality at 15 mM.

A number of in vitro studies of the effects of trichloroethylene and its metabolites on cardiac valve formation have been performed. The basic events of cardiac valve formation in mammals (including humans and laboratory animals) and birds are as follows:

1. Early in development (in utero or in ovo), the heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothelial cell layer. Between the two cell layers is extracellular matrix.
2. At a specific time in development, a subpopulation of endothelial cells lining the atrioventricular canal detaches from adjacent cells and invades the underlying extracellular matrix (Markwald et al. 1984). This event is termed an epithelial-mesenchymal cell transformation, when at least three distinct events occur: endothelial cell activation (chick stage 14), mesenchymal cell formation (chick stage 16), and mesenchymal cell invasion (migration) into the extracellular matrix (chick stages 17 and 18) (Boyer et al. 2000a).
3. Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and begin proliferating to populate the entire atrioventricular canal extracellular matrix.
4. The cardiac mesenchyme provides the cellular constituents for the septum intermedium and the valvular leaflets of the mitral (bicuspid) and tricuspid atrioventricular valves. The septum intermedium subsequently contributes to the lower portion of the interatrial septum and the membranous portion of the interventricular septum (Markwald et al. 1984, 1996; Boyer et al. 2000).

The chick stage 16 atrioventricular canal can be removed from the embryo and cultured in vitro on a three-dimensional hydrated collagen gel. During the 24 to 48 hours of incubation, all the stages described above occur in vitro and can be studied with or without test chemical exposures (e.g., Mjaatvedt et al. 1987, 1991; Loeber and Runyan 1990; Ramsdell and Markwald 1997). The in vitro model has identified a number of molecules as being involved with this transformation (e.g., fibronectin, laminin, galactosyltransferase [Mjaatvedt et al. 1997]; components of the extracellular matrix [Mjaatvedt et al. 1991]; and smooth muscle α-actin and transforming growth factor β3 [Nakajima et al. 1997; Ramsdell and Markwald 1997]).

Because trichloroethylene was implicated in heart defects of the chick (Bross et al. 1983), Boyer et al. (2000) explanted chick stage 16 atrioventricular canals onto gels with medium containing trichloroethylene at 50, 100, 150, 200, or 250 ppm. The explants were evaluated for epithelial-mesenchymal transformation, endothelial cell density, and immunohistochemistry.

Atrioventricular canal explants for chick stage 17 embryos were also cultured with no chemicals. Then, medium containing 0 or 250 ppm was added for 30 minutes, and the cell migration assay was performed. Trichloroethylene affected several elements of the epithelial-mesenchymal cell transformation process, including blockage of the endothelial cell-cell separation process that is associated with endothelial activation, inhibition of mesenchymal cell formation in a dose-dependent pattern, and no effect on the cell migration rate of the fully formed mesenchymal cells. The expression of three proteins selected as molecular markers of the epithelial-mesenchymal transformation was analyzed. Trichloroethylene inhibited the expression of transcription factor Mox-1 and extracellular matrix protein fibrillin 2 but had no effect on expression of smooth muscle α-actin. The authors suggested that trichloroethylene might cause cardiac valvular and septal malformations by inhibiting early endothelial separation and early events of mesenchymal cell formation in the embryonic heart (Boyer et al. 2000). Another interpretation (Hoffman et al. 2004) is that trichloroethylene affects the adhesive properties of endocardial cells. On the other hand, other have questioned the relevance of this study based on concerns that the concentrations used would not be tolerated by animals or achieved in humans (Dugard 2000). No direct experimental data are available that address trichloroethylene concentrations present in cardiac tissue in vivo.

Hoffman et al. (2004) proposed the using a whole embryo explant culture as a better system to evaluate the effects on the formation of the valves and septa of the heart, as anatomic relationships among tissues and organs are maintained and embryonic development can be monitored over the course of the experiment. Because mesenchymal cells first appear in the atrioventricular canal extracellular matrix at chick stage 16, they explanted stage-14 embryos for culture with trichloroethylene concentrations of 0, 10, 40, or 80 ppm. Only comparably staged and morphologically identical control and trichloroethylene-treated embryos were analyzed further by scanning laser confocal microscopy to assess cellular characteristics of the endocardial cushion tissues. With a trichloroethylene concentration of 80 ppm, there was a reduction (58.3% of the control value) in total cells of the atrioventricular cushion and an altered distribution of mesenchymal cells within the cushion. (Embryos treated with 40 ppm trichloroethylene were not assessed.) The authors also tested trichloroacetic acid in their whole embryo explant systems, and it too altered the distribution of cells in the endocardial cushions (Hoffman et al. 2004).

Using an in vitro mouse conceptus model in which haloacetic acids were added individually to culture medium, Hunter et al. (1996) showed that haloacetic acids generally are capable of causing altered development of the neural tube, eye and pharyngeal arches, and heart. With the exception of higher (≥250 μM) concentrations of monochloracetic acid, no increased embryo death was seen. Trichloroacetic acid was not teratogenic at 1,000 μM. At 2,000 μM, increased neural tube defects and fewer somites were observed. At 3,000 μM, an increase in eye, pharyngeal arch, and heart defects was seen. The cardiac anomalies were predominantly incomplete looping; a reduction in cardiac length beyond the bulboventricular fold and a reduction in the caliber of the heart tube lumen also were observed. Dichloroacetic acid was not teratogenic at 734 μM, but a significant, albeit inconsistent, decrease in somite number was variably observed at 1,468 μM or greater. Increased neural tube defects occurred at 5,871 μM, whereas pharyngeal defects and cardiac defects were observed at concentrations of 7,339 μM or greater. Extremely high concentrations (11,010 μM) caused rotational, eye, and somite dysmorphology. Virtually all haloacetic acids produced neural tube defects, but the potency varied by four orders of magnitude. The authors calculated benchmark concentrations for neural tube defects (defined as the lower 95% confidence interval of the concentration of acid required

to produce a 5% increase in the number of embryos with neural tube defects) of 91, 1,336, and 2,452 μM for monochloroacetic acid, trichloroacetic acid, and dichloroacetic acid, respectively. Generally, the chloroacetic acids were less potent than the bromoacetic acids but more potent than the fluoroacetic acids (Richard and Hunter 1996). This evidence that multiple halogenated compounds might be teratogenic supports the need for studies of outcomes after combined exposures.

Direct extrapolation of the results of direct embryo culture studies is limited because maternal absorption, excretion, and metabolism do not occur in in vitro systems. In addition, no conceptuses were exposed simultaneously to multiple haloacetic acids, all of which are frequently low-level water disinfection products, or to other common coexposure chemicals, including compounds metabolically upstream and downstream of the haloacetic acids, such as trichloroethylene and chloral hydrate. However, such models allow intrinsic toxicity to be evaluated.

Finally, one in vitro assay used bovine coronary endothelial cells cultured in medium containing 10% fetal bovine serum with antibiotics to suggest that endothelial nitric oxide synthase might be involved in trichloroethylene-mediated toxicity. Proliferating endothelial cells were treated with trichloroethylene at 0-100 μM and then stimulated with the calcium ionophore A23187 to determine changes in endothelial cells and endothelial nitric oxide synthase, nitric oxide, and superoxide anion generation. Trichloroethylene decreased concentrations of heat shock protein associated with endothelial nitric oxide synthase by 46.7% and inhibited vascular endothelial growth-factor-stimulated endothelial cell proliferation by 12% to 35%. These data show that trichloroethylene alters heat shock protein interactions with endothelial nitric oxide synthase and induces endothelial nitric oxide synthase to shift nitric oxide to superoxide-anion generation. The findings provide new insight into how trichloroethylene alters endothelial and endothelial nitric oxide synthase function to impair vascular endothelial growth-factor-stimulated endothelial proliferation. Such changes in endothelial function play an important role in the development of heart defects (Ou et al. 2003).

HUMAN STUDIES OF REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Currently, studies of the human reproductive and development effects of trichloroethylene consist of (1) retrospective, community-based studies of multiple pregnancy outcomes among residents of neighborhoods with varying documentation of trichloroethylene or trichloroethylene-related exposures; (2) studies of reproductive outcomes of men and women with nonquantitative occupational exposure to multiple, ill-defined organic solvents; (3) limited studies of health outcomes of children exposed to trichloroethylene, including intrauterine exposure; and (4) evaluations of spermatogenesis and sexual function among men with occupational exposure to high concentrations of trichloroethylene or trichloroethylene-related compounds. The following discussion provides a qualitative overview of the epidemiologic evidence. A more critical evaluation of relevant studies in terms of methods, exposures, and results is necessary to fully characterize the reproductive and developmental hazards of trichloroethylene (see Chapter 2 for guidance on how this should be done).

Community-Based Studies

Woburn, Massachusetts

Birth outcomes have been studied in communities of East Woburn, Massachusetts, that were served between 1964 and 1979 by wells contaminated with trichloroethylene (267 parts per billion [ppb]) and tetrachloroethylene (21 ppb). A health survey of 5,010 residents of Woburn (about 50% of the population) by Lagakos et al. (1986) found an increased likelihood of exposure to contaminated well water and ear and eye anomalies (odds ratio [OR] = 14.9; $P < 0.0001$) and perinatal deaths (OR = 10.0, $P = 0.003$) between 1970 and 1982. A combination of central nervous system, chromosomal, and oral cleft anomalies was also reported to be increased, but a review of data and the fact that this is an unconventional grouping of outcomes suggested that the finding was not plausibly related to exposure to the contaminated wells. Although no other birth defects or anomalies were reported, statistical power was limited. Spontaneous abortion and low birth weight were not increased; however, the study used a nonstandard cutoff weight to assess low birth weight (2,722 g versus 2,500 g).

A study by the Massachusetts Department of Public Health (MDPH/CDC/MHRI 1994) of the same population indicated the possibility of increased risk for small-for-gestational-age-babies in the context of exposure in the third trimester of pregnancy, particularly among teenage women (OR = 6.37; 95% confidence interval [CI] = 2.39, 16.99), and for preterm birth among older mothers with exposure in the third trimester (OR = 2.66; 95% CI = 1.14, 6.19). Others reported an interaction between maternal age and trichloroethylene (Yauck et al. 2004) and the similar compound tetrachloroethylene (Sonnenfeld et al. 2001) as well as other compounds (Fox et al. 1994; Jacobson et al. 1998). However, gestational age was not reported for more than half of the sample, making these observations unreliable.

The prevalence of structural birth defects was evaluated retrospectively between January 1975 and December 1984 and prospectively between January 1989 and March 1991. Over 4,500 hospital records were reviewed for the retrospective study, and over 11,000 for the prospective study. Ascertainment methods increased the possibility of a type II error for many birth defects, particularly congenital heart disease. The prevalence of choanal atresia (OR = 8.33, 95% CI = 2.37, 26.25; OR = 6.6, 95% CI = 1.99, 19.19) and hypospadias (OR = 1.59, 95% CI = 1.02, 2.45) was significantly higher in Woburn during the period of well contamination than in two national referent populations. Although the rates remained higher after well closure, the ascertainment methods for the post-well-closure period were more complete than during the contamination period. A referent population (such as from a retrospective analysis during the contamination years of the 12 noncontaminated communities used in the prospective study) was not included.

Camp Lejeune, North Carolina

Studies of developmental outcomes have been performed at the U.S. Marine Corps Base at Camp Lejeune, North Carolina, where drinking water was found to be contaminated with chlorinated volatile organic compounds, trichloroethylene, tetrachloroethylene, dichloroethylene, and lead. Exposure to these compounds was documented over a period of 34 months but likely occurred for years, perhaps as long as 30 years. Concentrations of trichloroethylene ranged from 8 to 1,400 ppb, dichloroethylene ranged from 12 to 407 ppb, and tetrachloroethylene ranged

from 76 to 215 ppb, depending on the water system and the time of testing. From the evaluations at Camp Lejuene to date, two potentially plausible findings appear. Trichloroethylene exposure appears to be associated with significantly smaller male infants, whether measured as a continuous variable or as a dichotomous variable (ATSDR 1998; Sonnenfeld et al. 2001). Among exposed male infants, adjusted mean birth weight was reduced by 312 g (90% CI = -540, -85; $P < 0.01$), and the prevalence of small for gestational age increased (OR = 3.9, 90% CI = 1.1, 11.9), whereas no difference was found in female infants.

Although such gender differences are not readily explained and have not been associated with trichloroethylene in other studies, male susceptibility has been seen with other chemicals, such as polychlorinated biphenyls and dioxins (Dewailly et al. 1993; Rylander et al. 1995). For tetrachloroethylene, two exposed subgroups appeared at greater risk of adverse outcomes: women over the age of 35 and those with a history of fetal loss (adjusted OR = 2.1, 90% CI = 0.9, 4.9; OR = 2.5; 90% CI = 1.5, 4.3). The adjusted differences in mean birth weight in the tetrachloroethylene-exposed infants in the two subgroups were -130 g (90% CI = -236, -23) and -104 g (90% CI = -174, -34), respectively. Increased environmental risk of birth defects among older women has been observed for trichloroethylene (Yauck et al. 2004), ethanol (Jacobson et al. 1996; Jacobson, et al. 1998), and smoking (Backe 1993; Fox et al. 1994). The association between prior fetal deaths and risk appeared to increase with the number of fetal deaths, increasing the probability that it was not a chance observation.

Limitations to the ATSDR (1998) study include the possibility of misclassification, particularly the possibility that unexposed mothers were included in the exposed population. This is more likely to be true in the tetrachloroethylene and long trichloroethylene exposed groups than in the short trichloroethylene exposed groups and would decrease the power to detect a difference and lead to a bias toward the null. The information about exposure for any individual is crude, as no information about water consumption was available, nor was information available about showering or other hot water activities, which would contribute to exposure by dermal and inhalation routes. Biologic monitoring information was also not available.

The clinical determination of gestational age from retrospective data is difficult and, in the ATSDR study, underestimates of gestational age likely occurred with birth weight used as a criterion because large-for-gestational-age preterm infants were removed from the study. Such an underestimate would decrease power and attenuate differences in the number of small-for-gestational-age infants between exposed and unexposed women. Removal of large-for-gestational-age preterm infants substantially decreased the number of preterm infants, which potentially decreased the power to detect a difference in prematurity rates. Data on tobacco and alcohol other important effect modifiers were not available. However, these exposures are less likely to have affected the exposure groups differentially.

ATSDR (2003) plans another study to assess birth defects and childhood cancer (leukemia, nonHodgkin s lymphoma) prevalence among children exposed to contaminated drinking water at Camp Lejeune. Surveys have been conducted to identify the study population and confirm the health outcomes reported by parents. A full study is planned to include all confirmed cases of birth defects and childhood cancers and an assessment of exposure to trichloroethylene and other drinking water contaminants by modeling the water system.

Santa Clara County, California

After the identification of well contamination with 1,1,1-trichloroethane, a solvent that shares some of the same principal metabolites as trichloroethylene (trichloroethanol and trichloroacetic acid), the public reported an increased number of spontaneous abortions and cases of congenital heart disease. A series of studies were done evaluating pregnancy outcomes (Deane et al. 1989; Wrensch et al. 1990a,b) and congenital heart disease (Swan et al. 1989). Deane et al. (1989) reported a higher rate of spontaneous abortions and congenital anomalies among exposed women (n = 250). The relative risk of congenital anomalies considered as a single entity was 3.1 (95 % CI = 1.1, 10.4). A later study by the same investigators (Wrensch et al. 1990a) expanded on this study and included an additional exposed area (n = 1,105). The analysis of the larger data set did not confirm the previous finding of an increase in spontaneous abortions in exposed women. An additional report (Wrensch et al. 1990b) that provided hydrogeologic assessment of the amount of exposure in two exposed census tracts found that the tract with higher concentrations of 1,1,1-trichloroethane had a lower rate of spontaneous abortions than the tract with lower 1,1,1-trichloroethane concentrations. The sample size was too small for statistical evaluation of birth defects.

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Three census tracts in Tucson, Arizona, (total population 1,099) were found to have trichloroethylene-contaminated well water between 1978 and 1981. Rodenbeck et al. (2000) estimated that concentrations of trichloroethylene in water ranged from less than 5 to 107 µg/L. Individual or household exposure could not be estimated because operational data were not available, so the entire population of all three tracts was considered evenly exposed. Mean exposure estimates were not given. Birth outcomes were compared between this group and contemporaneous births in other census tracts and for births in the census tracts after the exposure period (1983-1985). An association was reported between exposure to trichloroethylene via drinking water and very low birth weight (OR = 3.3; 95% CI = 0.5, 20.6). The authors suggested a similar association in the postexposure period; however, the magnitude was even smaller and less reliable (OR = 1.68, 95% CI = 0.41, 6.8). No relationship was seen between living in the exposed tracts and low birth weight or small-for-gestational-age babies. The problem of uncertain and uneven exposure is substantial and would decrease the power to

detect a difference. In addition, it is noteworthy that the exposure in this study was likely low compared with other population studies.

An increased frequency of congenital heart disease was suspected in Tucson, Arizona, in 1973. In 1981, drinking water contaminated with trichloroethylene (up to 270 ppb [approximately 0.009 mg/kg/day for a 60 kg adult], but also dichloroethylene and chromium) was detected in eight wells in Tucson Valley. In an epidemiologic study of children born between 1969 and 1987, Goldberg et al. (1990) noted that parents of children with congenital heart disease had a 3-fold greater likelihood of work or residence contact with the trichloroethylene-contaminated water area (n = 246/707, 35%) compared with parents of two control populations that had exposure rates of about 10%. The study has been criticized for inappropriate control groups, imprecision in determining exposure, and inclusion of years after the wells closed. Bove et al. (2002) reevaluated the data and restricted the analysis to the years when the wells were operational. In the reanalysis, the prevalence ratio of offspring cardiac defects among first-trimester exposed parents compared with that of unexposed parents was 2.58 (95% CI = 2.0, 3.4). Bove et al. (2002) also addressed the lack of exposure interviews for a large number of Goldberg et al. (1990) cases. Assuming that the noninterviewed cases and the interviewed cases had similar exposures or alternatively that the noninterviewed cases and the general Tucson population controls had similar exposures, the prevalence of cardiac defects in the exposed areas exceeded that in the uncontaminated areas by 2.3- and 2-fold, respectively. Thus, although the study by Goldberg et al. (1990) is flawed, additional analyses of the original data by an independent group of investigators yielded similar results and suggest an association between water contamination and congenital heart disease.

Milwaukee, Wisconsin

Yauck et al. (2004) performed a case-control study of 4,025 infants to evaluate the association between maternal residence close to trichloroethylene-emitting sites and infants with congenital heart defects in Milwaukee, Wisconsin. Mothers were categorized as older (older than 38 years) versus younger, exposed versus nonexposed, and presence versus absence of congenital heart defects. The proportion of mothers who were both older and had presumed trichloroethylene exposure was more than 6-fold greater among case infants (with congenital heart defects) than among control infants (3.3% versus 0.5%). When adjusted for other variables (e.g., race, ethnicity, maternal education, smoking), the risk of congenital heart defects was more than 3-fold greater among infants of older, exposed mothers than in infants of older, unexposed mothers (adjusted OR = 3.2; 95% CI = 1.2, 8.7). Older maternal age, alcohol use, chronic hypertension, and preexisting diabetes were each associated with increased incidence of congenital heart defects, but a residence close to trichloroethylene-emitting sites alone was not. The most common congenital heart defects were muscular ventricular septal defect (26.9%), secundum atrial septal defect (22.0%), membranous ventricular septal defect (20.8%), pulmonary stenosis (19.2%), and ventricular septal defect, not otherwise specified (15.5%). Maternal age was also an independent risk factor to other adverse birth outcomes, particularly chromosomal anomalies (e.g., Down s syndrome). Removing babies with any documented chromosomal abnormalities (n = 16) from the data set did not change the results of the logistic regression analysis.

Endicott, New York

The New York State Health Department in conjunction with ATSDR began an evaluation of health outcomes among residents living in areas of Endicott, New York, where soil vapor contamination with volatile organic compounds was identified (NYDOH 2005a). In the eastern study region, trichloroethylene was the most commonly found contaminant, occurring in indoor air at 0.18 to 140 $\mu g/m^3$, whereas reported soil values in some areas exceeded 10,000 $\mu g/m^3$. In the western study area, tetrachloroethylene was the most commonly found contaminant, ranging from 0.1 to 3.5 $\mu g/m^3$. The study years included 1978 to 2002 for the outcome variables birth weight and gestational age. Congenital anomalies were identified using the New York State Congenital Malformation Registry (data from 1983 to 2000). Individual information on each birth in the study and the comparison areas was used to estimate risk for each of the outcome variables, while controlling for maternal age, race, ethnicity, education and infant gender and year of birth.

When births (n = 1,440) in both study areas were considered together, the frequency of moderately low birth weight babies increased (standardized incidence rate [SIR] = 1.65 (95% CI = 1.00, 2.58) as well as term low-birth-weight births (SIR = 2.38; 95% CI = 1.10, 4.27). This observation was attributed to elevations observed in the eastern study region, the area with the greatest trichloroethylene contamination. In analyses that adjusted for multiple demographic factors, the relative risk of poor growth in the eastern study area was greater than in the controls. The ORs were 1.44 (95% CI = 1.13-1.83) and 1.79 (1.27-2.51) for low birth weight and term low birth weight, respectively. Among the congenital anomalies evaluated, the risk for all cardiac defects, as well as the subset of major cardiac defects, was elevated when both eastern and western areas were considered (adjusted rate ratio [RR] = 1.99; 95% CI = 1.27, 3.12; and RR = 2.62, 95% CI = 1.31, 5.23, respectively). Similar significant observations were seen for these end points when the eastern area was evaluated independently. The estimates from the data for the western study area were similar.

The evaluation of health effects at Endicott is an ongoing study and additional analyses and data refinements are planned. The current study is limited by the lack of individual exposure information, including concentration and duration of exposure. Birth defect cases were not validated by record review. Insufficient power was available to evaluate most birth defects. Finally, the quality of information for gestational age, a common problem with birth certificate data, was unclear but is needed for the subsequently planned study of small-for-gestational-age births.

Occupational Studies

Male Fertility

Bardodej and Vyskocil (1956) reported decreased libido in male workers exposed to trichloroethylene, but this effect did not appear to be related to any significant decrease in urinary excretion of adrenocorticosteroids. They gave no details about the control group, so the significance cannot be assessed. Sperm counts and morphology as well as Y chromosomal nondisjunction during spermatogenesis did not differ between male factory workers exposed to trichloroethylene at least 20 hours/week and physician controls (Rasmussen et al. 1988).

Recommendation: Additional studies to delineate subpopulations at greatest risk as well as to determine the mechanisms for the putative gender and maternal age-based susceptibility are warranted. Such interactions might be confirmed with analysis of existing epidemiologic data sets.

Cardiac Teratogenicity

Cardiac teratogenicity is the developmental end point in animal studies that has received the greatest attention. The committee is aware that considerable controversy has existed regarding cardiac teratogenesis, with some reviewers on both sides of the argument (Kaneko et al. 1997; Johnson et al. 1998b; Bove et al. 2002; Hardin et al. 2005). Multiple studies in several animal models, including mammalian (Smith et al. 1989, 1992; Epstein et al. 1992; Dawson et al. 1993; Drake et al. 2006) and avian (Bross et al. 1983; Loeber et al. 1988), suggest that trichloroethylene, or one or more of its metabolites (trichloroacetic acid and dichloroacetic acid), can cause cardiac teratogenesis. Of the studies performed, the avian studies are the most convincing, and mechanistic studies in birds have been performed. Although some rodent studies have shown effects (Smith et al. 1989, 1992; Dawson et al. 1993; Epstein et al. 1992), other studies have not (NTP 1985, 1986b; Fisher et al. 2001), suggesting either methodological or strain differences. The committee noted that the rodent studies showing trichloroethylene-induced cardiac teratogenesis at low doses were performed by investigators from a single institution. Also noted were the unusually flat dose-response curves in the low-dose studies from these investigators. For example, the incidences of heart malformations at trichloroethylene concentrations of 1.5 and 1,100 ppm (almost three orders of magnitude greater) were 8.2% to 9.2% (prepregnancy and during pregnancy) to 10.4% (during pregnancy only) (Dawson et al. 1993). The same pattern occurred with dichloroethylene. Thus, the animal data are inconsistent, and the apparent species differences have not been addressed.

Of the human epidemiologic studies, the Bove et al. (2002) reanalysis of the widely criticized, but positive, study by Goldberg et al. (1990) also found a positive association. Methodological problems limited the committee s consideration of the Santa Clara County data for congenital heart disease. The recent report of an increased incidence among residents of the Endicott, New York, area was also consistent with the Goldberg study. Of note, the effect size of a 2- to 3-fold increase in risk is similar across multiple studies. Plausibility for trichloroethylene-induced cardiac teratogenesis is increased by the fact that the most frequently observed cardiac defects in the human studies, those of the interventricular septae and the valves, are consistent with the most common defects seen in the animal studies. In addition, these specific defects are consistent with mechanistic studies demonstrating altered increased proliferation in the endocardial cushions at low dose (Drake et al. 2006) or alterations in endothelial cell activation and decreased expression of the transcription factor Mox-1 and extracellular matrix protein fibrillin 2, two markers of epithelial mesenchymal cell transformation, a key process in valve and septum formation (Boyer et al. 2000). Evidence that trichloroacetic acid and dichloroacetic acid are as potent as the parent compound suggests that CYP2E1 metabolic activation, as well as the fractional formation of trichloroacetic acid from chloral, is important in trichloroethylene cardiac teratogenesis.

Recommendations: Additional studies evaluating a lowest-observed-adverse-effect level and mode of action for trichloroethylene-induced developmental effects are needed to determine the most appropriate species for human modeling. More information is needed on metabolic activation in the avian model to evaluate interspecies differences, tissue-specific concentrations of trichloroethylene and its metabolites, and human data with better ascertainment of congenital heart disease and improved quantitative assessment of trichloroethylene exposures. Reanalysis, or perhaps additional data collection, from previous epidemiologic studies could be performed. For example, for some studies, more appropriate control data might be derived, which would cost-effectively improve the assessment of human trichloroethylene teratogenesis. The interaction of trichloroethylene with other solvents, some of which are known teratogens (e.g., ethanol and toluene), might also be pursued.

Reproductive Toxicity

On the basis of evidence generated by multiple authors in multiple rodent studies (Land et al. 1981; Kumar et al. 2000a; Forkert et al. 2002), the committee suggests that trichloroethylene is toxic to spermatogenesis and sperm fertilizing ability. However, whether these effects are transient or permanent is unclear. The mode of action is unclear and might or might not relate to hormonal alterations. Critical work by Berger and Horner (2003) demonstrated that trichloroethylene and tetrachloroethylene are not only male reproductive toxicants but also female reproductive toxicants in rats. Evidence for this finding included decreased sperm penetration and decreased fertilizability of oocytes from trichloroethylene- and tetrachloroethylene-treated females and reduced sperm plasma membrane protein binding to oocytes from trichloroethylene-treated females. Metabolic activation by CYP2E1 appears necessary for toxicity; however, which of the oxidative downstream metabolites is the proximate toxicant is not yet clear. The relevance of these trichloroethylene effects on male and female reproduction in animals to adverse reproductive outcomes in humans also is not clear.

Recommendations: More research is needed to better understand the effects of trichloroethylene on sperm and oocytes and possible consequences for reproduction. Mechanistic studies are needed to determine what metabolites are responsible for the effects.

HUMAN STUDIES OF REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Currently, studies of the human reproductive and development effects of trichloroethylene consist of (1) retrospective, community-based studies of multiple pregnancy outcomes among residents of neighborhoods with varying documentation of trichloroethylene or trichloroethylene-related exposures; (2) studies of reproductive outcomes of men and women with nonquantitative occupational exposure to multiple, ill-defined organic solvents; (3) limited studies of health outcomes of children exposed to trichloroethylene, including intrauterine exposure; and (4) evaluations of spermatogenesis and sexual function among men with occupational exposure to high concentrations of trichloroethylene or trichloroethylene-related compounds. The following discussion provides a qualitative overview of the epidemiologic evidence. A more critical evaluation of relevant studies in terms of methods, exposures, and results is necessary to fully characterize the reproductive and developmental hazards of trichloroethylene (see Chapter 2 for guidance on how this should be done).

Community-Based Studies

Woburn, Massachusetts

Birth outcomes have been studied in communities of East Woburn, Massachusetts, that were served between 1964 and 1979 by wells contaminated with trichloroethylene (267 parts per billion [ppb]) and tetrachloroethylene (21 ppb). A health survey of 5,010 residents of Woburn (about 50% of the population) by Lagakos et al. (1986) found an increased likelihood of exposure to contaminated well water and ear and eye anomalies (odds ratio [OR] = 14.9; $P < 0.0001$) and perinatal deaths (OR = 10.0, $P = 0.003$) between 1970 and 1982. A combination of central nervous system, chromosomal, and oral cleft anomalies was also reported to be increased, but a review of data and the fact that this is an unconventional grouping of outcomes suggested that the finding was not plausibly related to exposure to the contaminated wells. Although no other birth defects or anomalies were reported, statistical power was limited. Spontaneous abortion and low birth weight were not increased; however, the study used a nonstandard cutoff weight to assess low birth weight (2,722 g versus 2,500 g).

A study by the Massachusetts Department of Public Health (MDPH/CDC/MHRI 1994) of the same population indicated the possibility of increased risk for small-for-gestational-age-babies in the context of exposure in the third trimester of pregnancy, particularly among teenage women (OR = 6.37; 95% confidence interval [CI] = 2.39, 16.99), and for preterm birth

among older mothers with exposure in the third trimester (OR = 2.66; 95% CI = 1.14, 6.19). Others reported an interaction between maternal age and trichloroethylene (Yauck et al. 2004) and the similar compound tetrachloroethylene (Sonnenfeld et al. 2001) as well as other compounds (Fox et al. 1994; Jacobson et al. 1998). However, gestational age was not reported for more than half of the sample, making these observations unreliable.

The prevalence of structural birth defects was evaluated retrospectively between January 1975 and December 1984 and prospectively between January 1989 and March 1991. Over 4,500 hospital records were reviewed for the retrospective study, and over 11,000 for the prospective study. Ascertainment methods increased the possibility of a type II error for many birth defects, particularly congenital heart disease. The prevalence of choanal atresia (OR = 8.33, 95% CI = 2.37, 26.25; OR = 6.6, 95% CI = 1.99, 19.19) and hypospadias (OR = 1.59, 95% CI = 1.02, 2.45) was significantly higher in Woburn during the period of well contamination than in two national referent populations. Although the rates remained higher after well closure, the ascertainment methods for the post-well-closure period were more complete than during the contamination period. A referent population (such as from a retrospective analysis during the contamination years of the 12 noncontaminated communities used in the prospective study) was not included.

Camp Lejeune, North Carolina

Studies of developmental outcomes have been performed at the U.S. Marine Corps Base at Camp Lejeune, North Carolina, where drinking water was found to be contaminated with chlorinated volatile organic compounds, trichloroethylene, tetrachloroethylene, dichloroethylene, and lead. Exposure to these compounds was documented over a period of 34 months but likely occurred for years, perhaps as long as 30 years. Concentrations of trichloroethylene ranged from 8 to 1,400 ppb, dichloroethylene ranged from 12 to 407 ppb, and tetrachloroethylene ranged from 76 to 215 ppb, depending on the water system and the time of testing. From the evaluations at Camp Lejeune to date, two potentially plausible findings appear. Trichloroethylene exposure appears to be associated with significantly smaller male infants, whether measured as a continuous variable or as a dichotomous variable (ATSDR 1998; Sonnenfeld et al. 2001). Among exposed male infants, adjusted mean birth weight was reduced by 312 g (90% CI = -540, -85; $P < 0.01$), and the prevalence of small for gestational age increased (OR = 3.9, 90% CI = 1.1, 11.9), whereas no difference was found in female infants.

Although such gender differences are not readily explained and have not been associated with trichloroethylene in other studies, male susceptibility has been seen with other chemicals, such as polychlorinated biphenyls and

dioxins (Dewailly et al. 1993; Rylander et al. 1995). For tetrachloroethylene, two exposed subgroups appeared at greater risk of adverse outcomes: women over the age of 35 and those with a history of fetal loss (adjusted OR = 2.1, 90% CI = 0.9, 4.9; OR = 2.5; 90% CI = 1.5, 4.3). The adjusted differences in mean birth weight in the tetrachloroethylene-exposed infants in the two subgroups were -130 g (90% CI = -236, -23) and -104 g (90% CI = -174, -34), respectively. Increased environmental risk of birth defects among older women has been observed for trichloroethylene (Yauck et al. 2004), ethanol (Jacobson et al. 1996; Jacobson, et al. 1998), and smoking (Backe 1993; Fox et al. 1994). The association between prior fetal deaths and risk appeared to increase with the number of fetal deaths, increasing the probability that it was not a chance observation.

Limitations to the ATSDR (1998) study include the possibility of misclassification, particularly the possibility that unexposed mothers were included in the "exposed" population. This is more likely to be true in the tetrachloroethylene and "long trichloroethylene exposed" groups than in the "short trichloroethylene exposed" groups and would decrease the power to detect a difference and lead to a bias toward the null. The information about exposure for any individual is crude, as no information about water consumption was available, nor was information available about showering or other hot water activities, which would contribute to exposure by dermal and inhalation routes. Biologic monitoring information was also not available.

The clinical determination of gestational age from retrospective data is difficult and, in the ATSDR study, underestimates of gestational age likely occurred with birth weight used as a criterion because large-for-gestational-age preterm infants were removed from the study. Such an underestimate would decrease power and attenuate differences in the number of small-for-gestational-age infants between exposed and unexposed women. Removal of large-for-gestational-age preterm infants substantially decreased the number of preterm infants, which potentially decreased the power to detect a difference in prematurity rates. Data on tobacco and alcohol—other important effect modifiers—were not available. However, these exposures are less likely to have affected the exposure groups differentially.

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Tucson, Arizona

Three census tracts in Tucson, Arizona, (total population 1,099) were found to have trichloroethylene-contaminated well water between 1978 and 1981. Rodenbeck et al. (2000) estimated that concentrations of trichloroethylene in water ranged from less than 5 to 107 µg/L. Individual or household exposure could not be estimated because operational data were not available, so the entire population of all three tracts was considered evenly exposed. Mean exposure estimates were not given. Birth outcomes were compared between this group and contemporaneous births in other census tracts and for births in the census tracts after the exposure period (1983-1985). An association was reported between exposure to trichloroethylene via drinking water and very low birth weight (OR = 3.3; 95% CI = 0.5, 20.6). The authors suggested a similar association in the postexposure period; however, the magnitude was even smaller and less reliable (OR = 1.68, 95% CI = 0.41, 6.8). No relationship was seen between living in the exposed tracts and low birth weight or small-for-gestational-age babies. The problem of uncertain and uneven exposure is substantial and would decrease the power to detect a difference. In addition, it is noteworthy that the exposure in this study was likely low compared with other population studies.

An increased frequency of congenital heart disease was suspected in Tucson, Arizona, in 1973. In 1981, drinking water contaminated with trichloroethylene (up to 270 ppb [approximately 0.009 mg/kg/day for a 60 kg adult], but also dichloroethylene and chromium) was detected in eight wells in Tucson Valley. In an epidemiologic study of children born between 1969 and 1987, Goldberg et al. (1990) noted that parents of children with congenital heart disease had a 3-fold greater likelihood of work or residence contact with the trichloroethylene-contaminated water area (n = 246/707, 35%) compared with parents of two "control" populations that had exposure rates of about 10%. The study has been criticized for inappropriate control groups, imprecision in determining exposure, and inclusion of years after the wells closed. Bove et al. (2002) reevaluated the data and restricted the analysis to the years when the wells were operational. In the reanalysis, the prevalence ratio of offspring cardiac defects among first-trimester

"exposed" parents compared with that of "unexposed" parents was 2.58 (95% CI = 2.0, 3.4). Bove et al. (2002) also addressed the lack of exposure interviews for a large number of Goldberg et al. (1990) cases. Assuming that the noninterviewed cases and the interviewed cases had similar exposures or alternatively that the noninterviewed cases and the general Tucson population controls had similar exposures, the prevalence of cardiac defects in the exposed areas exceeded that in the uncontaminated areas by 2.3- and 2-fold, respectively. Thus, although the study by Goldberg et al. (1990) is flawed, additional analyses of the original data by an independent group of investigators yielded similar results and suggest an association between water contamination and congenital heart disease.

Milwaukee, Wisconsin

Yauck et al. (2004) performed a case-control study of 4,025 infants to evaluate the association between maternal residence close to trichloroethylene-emitting sites and infants with congenital heart defects in Milwaukee, Wisconsin. Mothers were categorized as older (older than 38 years) versus younger, exposed versus nonexposed, and presence versus absence of congenital heart defects. The proportion of mothers who were both older and had presumed trichloroethylene exposure was more than 6-fold greater among case infants (with congenital heart defects) than among control infants (3.3% versus 0.5%). When adjusted for other variables (e.g., race, ethnicity, maternal education, smoking), the risk of congenital heart defects was more than 3-fold greater among infants of older, exposed mothers than in infants of older, unexposed mothers (adjusted OR = 3.2; 95% CI = 1.2, 8.7). Older maternal age, alcohol use, chronic hypertension, and preexisting diabetes were each associated with increased incidence of congenital heart defects, but a residence close to trichloroethylene-emitting sites alone was not. The most common congenital heart defects were muscular ventricular septal defect (26.9%), secundum atrial septal defect (22.0%), membranous ventricular septal defect (20.8%), pulmonary stenosis (19.2%), and ventricular septal defect, not otherwise specified (15.5%). Maternal age was also an independent risk factor to other adverse birth outcomes, particularly chromosomal anomalies (e.g., Down's syndrome). Removing babies with any documented chromosomal abnormalities (n = 16) from the data set did not change the results of the logistic regression analysis.

Endicott, New York

The New York State Health Department in conjunction with ATSDR began an evaluation of health outcomes among residents living in areas of Endicott, New York, where soil vapor contamination with volatile organic

compounds was identified (NYDOH 2005a). In the eastern study region, trichloroethylene was the most commonly found contaminant, occurring in indoor air at 0.18 to 140 $\mu g/m^3$, whereas reported soil values in some areas exceeded 10,000 $\mu g/m^3$. In the western study area, tetrachloroethylene was the most commonly found contaminant, ranging from 0.1 to 3.5 $\mu g/m^3$. The study years included 1978 to 2002 for the outcome variables birth weight and gestational age. Congenital anomalies were identified using the New York State Congenital Malformation Registry (data from 1983 to 2000). Individual information on each birth in the study and the comparison areas was used to estimate risk for each of the outcome variables, while controlling for maternal age, race, ethnicity, education and infant gender and year of birth.

When births (n = 1,440) in both study areas were considered together, the frequency of moderately low birth weight babies increased (standardized incidence rate [SIR] = 1.65 (95% CI = 1.00, 2.58) as well as term low-birth-weight births (SIR = 2.38; 95% CI = 1.10, 4.27). This observation was attributed to elevations observed in the eastern study region, the area with the greatest trichloroethylene contamination. In analyses that adjusted for multiple demographic factors, the relative risk of poor growth in the eastern study area was greater than in the controls. The ORs were 1.44 (95% CI = 1.13-1.83) and 1.79 (1.27-2.51) for low birth weight and term low birth weight, respectively. Among the congenital anomalies evaluated, the risk for all cardiac defects, as well as the subset of major cardiac defects, was elevated when both eastern and western areas were considered (adjusted rate ratio [RR] = 1.99; 95% CI = 1.27, 3.12; and RR = 2.62, 95% CI = 1.31, 5.23, respectively). Similar significant observations were seen for these end points when the eastern area was evaluated independently. The estimates from the data for the western study area were similar.

The evaluation of health effects at Endicott is an ongoing study and additional analyses and data refinements are planned. The current study is limited by the lack of individual exposure information, including concentration and duration of exposure. Birth defect cases were not validated by record review. Insufficient power was available to evaluate most birth defects. Finally, the quality of information for gestational age, a common problem with birth certificate data, was unclear but is needed for the subsequently planned study of small-for-gestational-age births.

Occupational Studies

Male Fertility

Bardodej and Vyskocil (1956) reported decreased libido in male workers exposed to trichloroethylene, but this effect did not appear to be related

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Key Scientific Issues

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